Exhibit "B"
To Applicant's Response to
Office Action of 12 February 2003
for US Patent Application No. 09/982,018

Metabolism of 20(*S*)- and 20(*R*)-Ginsenoside R_{g3} by Human Intestinal Bacteria and Its Relation to *in Vitro* Biological Activities

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When ginsenoside R_{g3} was anaerobically incubated with human fecal microflora, all specimens metabolized ginsenoside R_{g3} to ginsenoside R_{b2} and protopanaxadiol. The main metabolite was ginsenoside R_{b2}. 20(*S*)-ginsenoside R_{g3} was quickly transformed to 20(*S*)-ginsenoside R_{b2} or 20(*S*)-protopanaxadiol in an amount 19-fold that compared with the transformation of 20(*R*)-ginsenoside R_{g3} to 20(*R*)-ginsenoside R_{b2} or 20(*R*)-protopanaxadiol. Among the bacteria isolated from human fecal microflora, *Bacteroides* sp., *Eubacterium* sp., and *Bifidobacterium* sp. metabolized ginsenoside R_{g3} to protopanaxadiol *via* ginsenoside R_{b2}. However, *Fusobacterium* sp. metabolized ginsenoside R_{g3} to ginsenoside R_{b2} alone. Among ginsenoside R_{g3} and its metabolites, 20(*S*)-protopanaxadiol and 20(*S*)-ginsenoside R_{b2} exhibited the most potent cytotoxicity against tumor cell lines, 20(*S*)- and 20(*R*)-protopanaxadiols potently inhibited the growth of *Helicobacter pylori*, and 20(*S*)-ginsenoside R_{b2} inhibited H^+/K^+ ATPase of rat stomach.

Key words ginsenoside R_{g3}; intestinal bacteria; ginsenoside R_{b2}; protopanaxadiol; cytotoxicity; *Helicobacter pylori*

Most herbal medicines are orally administered and their components inevitably come into contact with intestinal microflora in the alimentary tract. These components may be transformed before they are absorbed from the gastrointestinal tract. Studies on the metabolism of herbal medicine components by human intestinal microflora are therefore of great importance in understanding their biological effects.[1,2]

Among herbal medicines, ginseng (the root of *Panax ginseng* C.A. MEYER, Araliaceae) is frequently used in Asian countries as a traditional medicine. The major components of ginseng are ginsenosides.[3,4] These ginsenosides have been reported to show various biological activities including anti-inflammatory activity[5] and antitumor effects.[6,7] The pharmacological actions of these ginsenosides has been explained by the biotransformation of ginsenosides by human intestinal bacteria.[8–12] Transformed 20-*O*-β-D-glucopyranosyl-20(*S*)-protopanaxadiol (IH-901, compound K) from ginsenosides R_{b1}, R_{b2} and R_c induces an antimetastatic or anticarcinogenic effect.[13–15] In addition, ginsenosides R_{b1}, R_{b2}, and R_c are transformed to ginsenoside R_{g3} by treatment with mild acid such as stomach acid.[16] Furthermore, ginsenoside R_{g3} is a main component of Red Ginseng.[17] However, studies on the metabolism of ginsenoside R_{g3} by human intestinal bacteria are not complete.

Therefore we investigated the human intestinal bacteria capable of metabolizing ginsenoside R_{g3} and its related biological activities, such as *in vitro* cytotoxicity and anti-*Helicobacter pylori* (HP) activity.

MATERIALS AND METHODS

Materials and Bacterial Strains Sodium thioglycolate and ascorbic acid were purchased from Sigma Chemical Co. (U.S.A.). General anaerobic medium (GAM) was purchased from Nissui Pharmaceutical Co., Ltd., (Japan). Tryptic soy broth was purchased from Difco Co. (U.S.A.). *p*-Nitrophenyl β-D-glucopyranoside (PNG) was purchased from Sigma (U.S.A.). The other chemicals were of analytical reagent grade.

Tumor cell lines were purchased from the Korean Cell Bank. HP ATCC43504 was purchased from ATCC, HP NCTC11638 was purchased from NCTC. HP82516 and HP4 clinically isolated from Korean gastroscopic samples were used. They were inoculated onto brucella agar plates supplemented with 7% horse serum and cultured for 3 days at 37 °C under microaerophilic conditions (AnaeroPak Campylo: 85% N_2, 10% CO_2, and 5% O_2).

Isolation of Transformants of Ginsenoside R_{b1} under Mild Acidic Conditions Ginsenoside R_{b1} (2 g) was treated in mild acidic conditions[16] at 37 °C for 2 h, as previously reported, concentrated at 60 °C and extracted with *n*-BuOH. From this BuOH fraction, 20(*S*)- and 20(*R*)-ginsenoside R_{g3} were isolated according to the previous method.[18] The BuOH fraction was chromatographed on a silica gel column using $CHCl_3$–MeOH–H_2O (10 : 3 : 1, lower layer) to produce Δ^{20}-ginsenoside R_{g3} (0.05 g), and *isomeric* 20(*S*)- and 20(*R*)-ginsenoside R_{g3} (0.6 g). The *isomeric* mixture was dissolved in pyridine, and acetic anhydride was added drop by drop in ice bath and stirred at room temperature for 10 h. The reaction mixture was dispersed in ice water and extracted three times with EtOAc. The organic layer was washed with 5% HCl, saturated aqueous $NaHCO_3$, and brine, followed by drying over anhydrous magnesium sulfate. The residue was applied to silica gel column chromatography and eluted with dichloromethane–EtOAc (8 : 1) to afford peracetylated-20(*S*)-ginsenoside R_{g3} and peracetylated-20(*R*)-ginsenoside R_{g3}. These compounds were each dissolved in 5% NaOH/*n*-BuOH (20 ml) in an ice bath and stirred at room temperature overnight. After the reaction mixtures were washed with water and evaporated to dryness, the residues were applied to silica gel column chromatography using $CHCl_3$–MeOH–H_2O (9 : 3 : 1, v/v) to give 20(*S*)-ginsenoside R_{g3} (0.21 g) and 20(*R*)-ginsenoside R_{g3} (0.12 g), respectively.

20(*S*)-Ginsenoside R_{g3} (**4**): Colorless needles, mp 248—250 °C (dec.), FAB-MS (*m/z*) 786 $[M+1]^+$.

20(*R*)-Ginsenoside R_{g3} (**5**): Colorless needles, mp 299—302 °C (dec.), FAB-MS (*m/z*) 786 $[M+1]^+$.

Isolation of Metabolites of Ginsenoside R_{g3} by Human Intestinal Bacteria The reaction mixture containing 100 mg of 20(*S*)-ginsenoside R_{g3} (or 20(*R*)-ginsenoside R_{g3}) and

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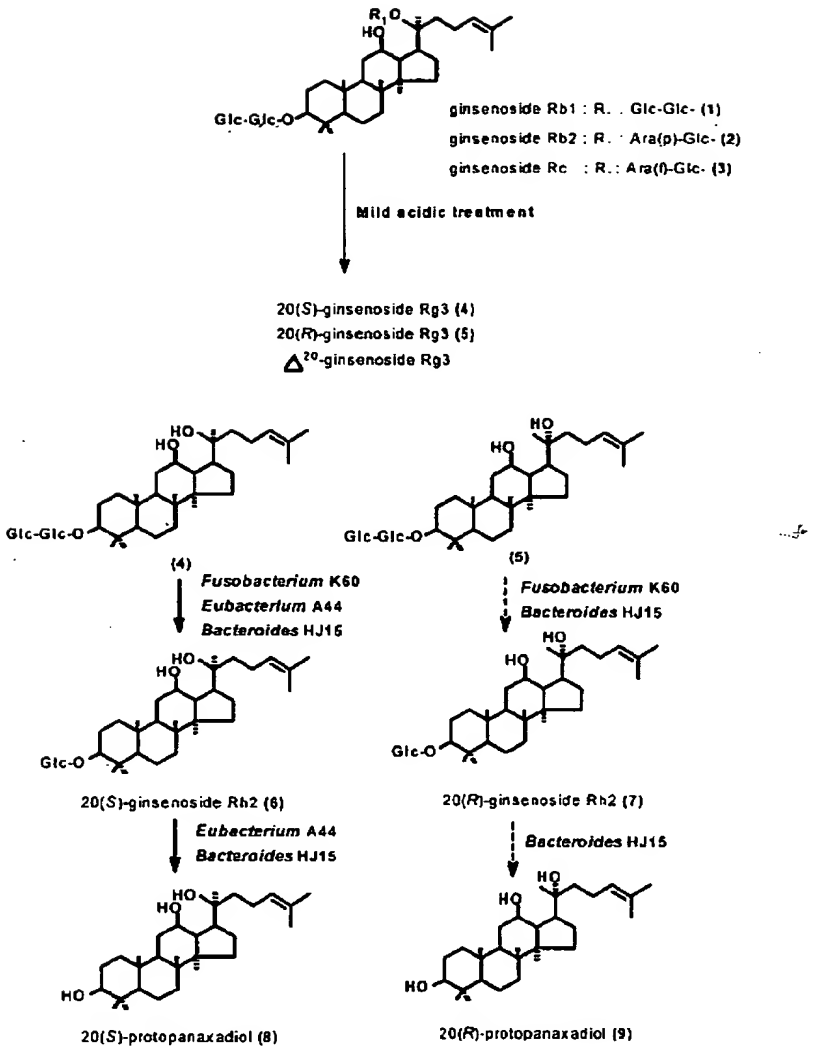

Chart 1. Proposed Metabolic Pathway of Ginsenoside R_{g3} by Human Intestinal Bacteria

Each metabolic pathway was potently catalyzed by the bacteria listed from bacteria tested in this experiment. →, main pathway; ⇢, minor pathway.

500 mg fresh fecal suspension was incubated for 24 h (or 72 h for 20(*R*)-ginsenoside R_{g3}) at 37 °C. The reaction mixture was adjusted to pH 2 with HCl, extracted with ethylacetate, evaporated to dryness, and applied to silica gel column chromatography; solvent, $CHCl_3$–MeOH–H_2O (65 : 35 : 10 v/v, lower phase). 20(*S*)-ginsenoside R_{h2} (12 mg) and 20(*R*)-ginsenoside R_{h2} (20 mg), and 20(*S*)-protopanaxadiol (15 mg) and 20(*R*)-protopanaxadiol (5 mg) were isolated.

Ten grams of fresh feces were suspended with 100 ml of anaerobic diluted media and centrifuged at 200×***g*** for 5 min, and the precipitate was discarded. The supernatant was centrifuged at 10000×***g*** for 20 min. The precipitate was washed twice with saline and used in the experiment.

20(*S*)-Ginsenoside R_{h2} (**6**): Colorless needles, mp 219—221 °C (dec.), FAB-MS (*m/z*) 623 $[M+1]^+$.

20(*R*)-Ginsenoside R_{h2} (**7**): Colorless crystals, mp 208—210 °C (dec.), FAB-MS (*m/z*) 623 $[M+1]^+$.

20(*S*)-protopanaxadiol (**8**): Colorless needles, mp 198—200 °C (dec.), Electron-impact (EI)-MS (*m/z*) 459 $[M]^+$.

20(*R*)-protopanaxadiol (**9**): White needless, mp 236—238 °C (dec.), EI-MS (*m/z*) 459 $[M]^+$.

Screening of Bacteria Metabolizing Ginsenoside R_{g3} Among human feces tested, potent ginsenoside R_{g3}-hydrolyzing fresh feces were anaerobically diluted 10^3- to 10^7-fold. Two hundred microliters of the diluted fecal suspension were inoculated on GAM agar plates. The plates were anaerobically incubated at 37 °C for 24 h. More than 100 colonies isolated from several plates, or bacteria previously isolated from human intestinal bacteria (*Bacteroides* HJ-15, *Bacteroides* JY-6, *Eubacterium* A-44, *Bifidobacterium* K-111, and *Fusobacterium* K-60)[13] were cultured in 50 ml of tryptic soy broth containing 0.01% sodium thioglycolate and 0.1% ascorbic acid (TSTA), and then each cultured cell was centrifuged at 3000×***g*** for 10 min and washed twice with saline. The activities of these collected cells in metabolizing ginsenoside R_{g3} were measured using the assay method described.

Assay of Metabolic Activity of Ginsenoside R_{g3} by Human Intestinal Bacteria The reaction mixture containing 100 μl of 1 mM ginsenoside R_{g3} and 100 μl of fecal sus-

pension (or bacterial suspension cultured in TSTA broth) was incubated for 12 h at 37 °C. The reaction mixture was adjusted to pH 2 with HCl, extracted with ethylacetate, evaporated, and assayed by TLC: TLC plates, Silica gel $60F_{254}$ (Merck Co., U.S.A.); developing solvent, $CHCl_3$–MeOH–H_2O (65 : 35 : 10 v/v, lower phase). The plates were stained by spraying with MeOH–H_2SO_4 (95 : 5 v/v), followed by heating. The stained TLC plates were then analyzed using a TLC scanner (Shimadzu model CS-9301PC, Japan).

Each isolated bacterium was cultured in 50 ml of TSTA broth and centrifuged at 3000×*g* for 30 min. Each collected bacterial pellet was suspended in 50 mM phosphate buffer and used as a crude enzyme solution.

Assay of β-Glucosidase Activity β-Glucosidase enzyme activity was assayed according to our previous method.[19]

Time Course of the Metabolism of Ginsenoside R_{g3} by Human Fecal Microflora Ginsenoside R_{g3}-metabolizing activity was measured as follows. Two milliliters of fresh human fecal suspension (250 mg/ml) or the isolated intestinal bacterial suspension (250 mg/ml or 1 g/ml) were added to 8 ml of anaerobic diluted medium[11] containing 0.3 mM 20(*S*)-ginsenoside R_{g3} (or 20[*R*]-ginsenoside R_{g3}) and then was incubated at 37 °C for 1 d, and an aliquot (0.5 ml) of the reaction mixture was periodically extracted twice with 1 ml of ethylacetate. The ethylacetate fraction was analyzed by TLC. Ginsenoside R_{g3} and its metabolites were identified and assayed against authentic compounds isolated according to our previous method.

The fresh feces of a healthy volunteer (2 g) were collected, suspended in 48 ml of anaerobic dilution medium, centrifuged at 200×*g* and the supernatant was centrifuged at 10000×*g* for 30 min.

The isolated bacteria were cultured in 500 ml of TSTA broth and centrifuged at 10000×*g* for 30 min and washed with the anaerobic dilution medium. The fecal and bacterial precipitates (250 mg) were resuspended in 1.75 ml of anaerobic dilution medium.

Assay of Anti-HP Activity A growth inhibition assay of HP was performed according to the previous method.[19]

Preparation and Assay of HP Urease The preparation of partially purified urease from HP was performed according to our previous method.[19] Urease activity was determined by the indophenol method.[20] Acetohydroxamic acid was used as a positive control.

Preparation and Assay of Rat Stomach H^+/K^+-ATPase Gastric H^+/K^+-ATPase was partially purified from the parietal cell-rich fraction of male Sprague-Dawley rat (200—250 g) stomach as described by Saccomani and Mukidjam.[21]

Rat gastric H^+/K^+-ATPase activity was also determined according to the modified method of Saccomani and Mukidjam.[21]

***In Vitro* Cytotoxicity Assay** The *in vitro* cytotoxicity was tested against L1210 (mouse lymphocytic leukemia cell line), P388 (mouse lymphoid neoplasma cell line), A549 (human lung carcimoma), and Me180 (human cervix uterine carcinoma) cell lines by MTT [3-(3,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay according to the method of Carmichael *et al.*[22]

RESULTS

Metabolism of Ginsenoside R_{g3} by Human Intestinal Microfl ra When ginsenoside R_{b1} decomposed under mild acidic conditions (0.1 N HCl), two additional spots were observed on TLC. They were tentatively designated as Δ^{20}-ginsenoside R_{g3} and isomeric ginsenoside R_{g3} in the order of increasing polarity. The isomeric ginsenoside was separated into 20(*S*)- and 20(*R*)-ginsenoside R_{g3} by acetylation and solubility. The acid-labile nature of ginsenosides was observed even if the concentration of HCl was as low as 0.01 N HCl. These results suggest that this reaction of ginsenoside R_{g3} could occur in the stomach or during the fermentation of ginseng.

Therefore, to investigate the metabolic process of ginsenside R_{g3} before absorption in the intestine, 20(*S*)- and 20(*R*)-ginsenside R_{g3} were incubated with human fecal suspension. 20(*S*)-Ginsenoside R_{h2} and 20(*S*)-protopanaxdiol, and 20(*R*)-protopanaxdiol and 20(*R*)-ginsenoside R_{h2} were observed as the metabolites, respectively. The main metabolites of these compounds were 20(*S*)- and 20(*R*)-ginsenoside R_{h2} during 24-h incubation, respectively.

When 20(*S*)- and 20(*R*)-ginsenoside R_{g3}-hydrolyzing activity was first assayed in fecal specimens from five different human subjects, their transforming activities were detected in all specimens. However, these activities varied depending on the individual samples. The mean of the activities transforming 20(*S*)- and 20(*R*)-ginsenoside R_{g3} to 20(*S*)- and 20(*R*)-ginsenoside R_{h2} were 0.57±0.20 and 0.03±0.002 nmol/h/mg wet weight of feces, respectively (Fig. 1). The metabolic activity of 20(*S*)-ginsenoside R_{g3} to 20(*S*)-ginsenoside R_{h2} was 19-fold higher than that of 20(*R*)-ginsenoside R_{g3} to 20(*R*)-ginsenoside R_{h2}. When 20(*S*)-ginsenoside R_{g3} was incubated with human fecal microflora, it began to be transformed to 20(*S*)-ginsenoside R_{h2}, which was transformed to 20(*S*)-protopanaxadiol (Fig. 2). However, 24 h after incubation the main metabolite was 20(*S*)-ginsenoside R_{h2}. The metabolic pathway of 20(*R*)-ginsenoside R_{g3} was similar to that of 20(*S*)-ginsenoside R_{g3}. However, its metabolism was weakly

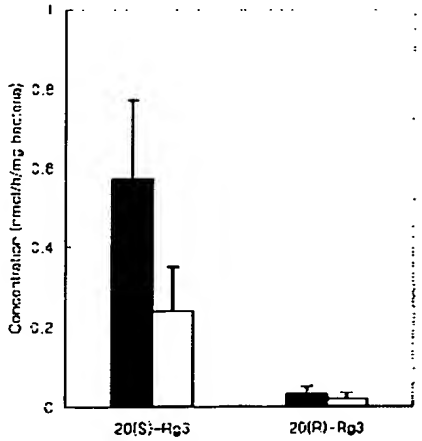

Fig. 1. The Activity Transforming Ginsenoside R_{g3} to Ginsenoside R_{h2} and Protopanaxadiol by Human Fecal Microflora

Human fecal suspension was prepared and its activity was assayed as described in Materials and Methods. Transforming activity of ginsenoside R_{g3} to ginsenoside R_{h2} was calculated from total transformed ginsenoside R_{h2} and protopanaxadiol. Symbols indicate the following: ■, activity transforming ginsenoside R_{g3} to ginsenoside R_{h2}; □, activity transforming ginsenoside R_{g3} to protopanaxadiol.

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β-Glucosidase Activities f Human Intestinal Bacteria We cultured more than 30 bacteria isolated from human feces and measured their β-glucosidase activities to evaluate the glycoside-metabolizing activity of the bacteria isolated from human feces (Table 1). Using PNG as a substrate, most bacteria produced potent β-glucosidase activity. These bacteria were also capable of hydrolyzing 20(*S*)-ginsenoside R_{g3} mainly giving 20(*S*)-ginsenoside R_{h2}. The most potently 20(*S*)-ginsenoside R_{g3}-hydrolyzing bacteria were *Bacteroides* HJ-15, *Bifidobacterium* K-111, *Eubacterium* A-44, and *Fusobacterium* K-60. These bacteria, except for *Fusobacterium* K-60, transformed 20(*S*)-ginsenoside R_{g3} to 20(*S*)-protopanaxdiol *via* 20(*S*)-ginsenoside R_{h2} (Fig. 3). However, 20(*R*)-ginsenoside R_{g3} was hydrolyzed very little by most intestinal bacteria except for *Bacteroides* HJ-15 and *Fusobacterium* K-60. Nevertheless, these ginsenoside R_{g3}-transforming bacteria also hydrolyzed PNG, a synthetic substrate of β-glucosidase, but PNG-hydrolyzing activity was not proportional to ginsenoside R_{g3}-hydrolyzing activity.

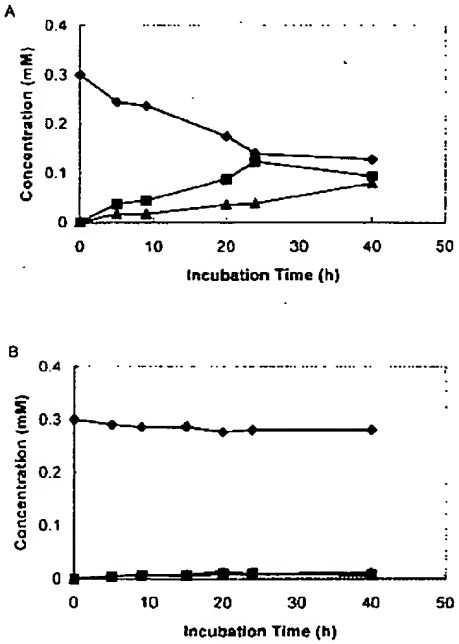

Fig. 2. Time Course of 20(*S*)- and 20(*R*)-Ginsenoside R_{g3} Transformation by Human Fecal Microflora

Human fecal suspension was prepared and their metabolites were assayed as described in Materials and Methods. A, 20(*S*)-ginsenoside R_{g3}; B, 20(*R*)-ginsenoside R_{g3}. ◆, 20(*S*)- or 20(*R*)-ginsenoside R_{g3}; ■, 20(*S*)- or 20(*R*)-ginsenoside R_{h2}; ▲, 20(*S*)- or 20(*R*)-protopanaxadiol.

Biological Activities of Ginsenoside R_{g3} and Its Metabolites The inhibitory effect of ginsenoside R_{g3} and its metabolites on the growth of HP was measured (Table 2). Ginsenosides R_{b1}, R_{g3}, and R_{h2} did not inhibit HP growth. However, 20(*S*)- and 20(*R*)-protopanaxadiols inhibited HP growth at MICs of 50—100 μg/ml. The inhibitory effects of these compounds on the activity of HP urease and H^+/K^+ ATPase were also measured (Table 3). Most of the tested compounds did not inhibit these enzymes. However, 20(*S*)-ginsenosides R_{g3} and R_{h2} weakly inhibited H^+/K^+ ATPase of the rat stomach, with their IC_{50} values of 0.6 and 0.48 mg/ml, respectively.

We also investigated the *in vitro* cytotoxic activity of ginsenoside R_{g3} and its metabolites on the tumor cell lines (Table 4). Ginsenoside R_{b1} and 20(*R*)-ginsenoside R_{g3} did not exhibit cytotoxicity against the tumor cell lines. 20(*S*)-Ginsenoside R_{g3} only exhibited weak cytotoxicity. However, the metabolites of 20(*S*)-ginsenoside R_{g3}, 20(*S*)-ginsenoside R_{h2} and 20(*S*)-protopanaxadiol, showed potent cytotoxicity against tumor cell lines, with IC_{50} values of 22—33 and 18—33 μM, respectively.

DISCUSSION

The main components of ginseng, which is frequently used in Asia, are ginsenoside R_{b1} and R_{b2}. These ginsenosides are likely transformed to 20-β-*O*-glucopyranosyl-20(*S*)-protopanaxadiol (IH-901) or 20(*S*)-protopanaxadiol *via* ginsenoside R_d or gypenoside XVII by human intestinal bacteria.[11,13,23]

Han *et al.* reported that ginsenoside R_{b1} and R_{b2} were transformed to ginsenoside R_{g3} when these compounds were incubated in mildly acidic conditions,[16] and suggested that this transformation of ginsenosides R_{b1} and R_{b2} to ginsenoside R_{g3} could occur in the stomach. In addition, ginsenoside R_{g3} is a major component of Red Ginseng rather than of Ginseng.[17] When ginsenoside R_{g3} was incubated with human fecal microflora, it was transformed to ginsenoside R_{h2} and protopanaxadiol. When 20(*S*)-ginsenoside R_{g3} was incubated

Table 1. β-Glucosidase Activity of Representative Intestinal Bacteria Isolated from Human Feces

	PNG (nmol/(min·mg))	Transforming activity[a] (nmol/(h·mg))			
		20(*S*)-Ginsenoside R_{g3}		20(*R*)-Ginsenoside R_{g3}	
		20(*S*)-R_{h2}	20(*S*)-Ppd	20(*R*)-R_{h2}	20(*R*)-Ppd
Bacteroides HJ15	1.191	1.455	0.002	0.06	0.01
Bacteroides JY6	0.242	0.06	0.01	<0.01	<0.01
Bifidobacterium K-111	0.620	0.102	0.010	0	0
Escherichia coli HGU-3	0.022	0	0	0	0
Eubacterium A44	0.451	0.371	0.149	<0.01	<0.01
Eubacterium L8	0.104	0.106	<0.01	0	0
Fusobacterium K-60	0.249	0.759	0	0.02	0
Streptococcus S2	0.100	0.039	0	0	0
Streptococcus S10	0.129	0.032	<0.01	<0.01	<0.01

a) Transforming activity of ginsenoside R_{g3} to ginsenoside R_{h2} was calculated from total transformed ginsenoside R_{h2} and protopanaxadiol.

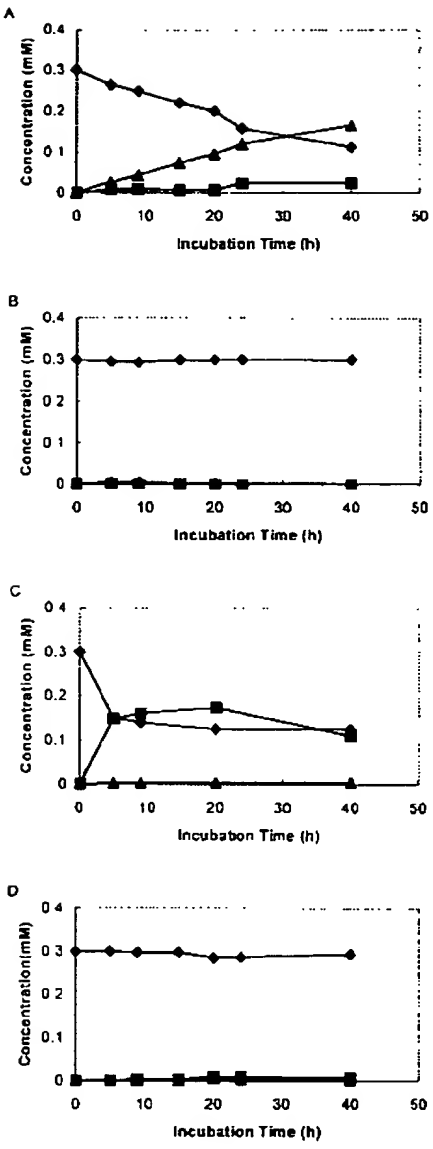

Fig. 3. Time Course of 20(*S*)- and 20(*R*)-Ginsenoside R_{g3} Transformation by Human Intestinal Bacteria *Eubacterium* A-44 and *Fusobacterium* K-60

The intestinal bacterial suspension was prepared and their metabolites were assayed as described in Materials and Methods. A, 20(*S*)-Ginsenoside R_{g3} was treated with *Eubacterium* A-44 (1 g); B, 20(*R*)-Ginsenoside R_{g3} was treated with *Eubacterium* A-44 (1 g); C, 20(*S*)-Ginsenoside R_{g3} was treated with *Fusobacterium* K-60 (250 mg); D, 20(*R*)-Ginsenoside R_{g3} was treated with *Fusobacterium* K-60 (250 mg). ◆, 20(*S*)- or 20(*R*)-ginsenoside R_{g3}; ■, 20(*S*)- or 20(*R*)-ginsenoside R_{h2}; ▲, 20(*S*)- or 20(*R*)-protopanaxadiol.

with intestinal bacterial strains previously isolated, it was mainly transformed to ginsenoside R_{h2}. However, 20(*S*)-ginsenoside R_{g3} was potently metabolized to 20(*S*)-ginsenoside R_{h2} and 20(*S*)-protopanaxadiol, while 20(*R*)-ginsenoside R_{g3} was barely metabolized by the human fecal microflora and intestinal bacterial strains. Most of the isolated intestinal bacteria hydrolyzed PNG. However, some intestinal bacteria only could convert ginsenoside R_{g3} to protopanaxadiol *via* ginsenoside R_{h2}. This suggests that 20(*S*)-ginsenoside R_{g3} is not only a good substrate of β-glucosidase of intestinal bacteria, but is also highly soluble in water compared with 20(*R*)-ginsenoside R_{g3}.

Table 2. Anti-*Helicobacter pylori* Activity of 20(*S*)- and 20(*R*)-Ginsenoside R_{g3} and Their Metabolites

Compound	MIC (μg/ml)			
	HP ATCC43504	HP NCTC11638	HP 82516	HP 4
Ginsenoside R_{b1}	>100	>100	>100	>100
20(*S*)-Ginsenoside R_{g3}	>100	>100	>100	>100
20(*R*)-Ginsenoside R_{g3}	>100	>100	>100	>100
20(*S*)-Ginsenoside R_{h2}	>100	>100	>100	>100
20(*R*)-Ginsenoside R_{h2}	>100	>100	>100	>100
20(*S*)-Protopanaxadiol	50	50	50	50
20(*R*)-Protopanaxadiol	50	50	100	100

Table 3. Inhibitory Effects of Ginsenoside R_{g3} and Its Metabolites on HP Urease and Rat Stomach H^+/K^+ ATPase

Compound	IC_{50} (mg/ml)	
	HP urease	Stomach H^+/K^+ ATPase
Ginsenoside R_{b1}	>1	>1
20(*S*)-Ginsenoside R_{g3}	>1	0.6
20(*R*)-Ginsenoside R_{g3}	>1	>1
20(*S*)-Ginsenoside R_{h2}	>1	0.48
20(*R*)-Ginsenoside R_{h2}	>1	>1
20(*S*)-Protopanaxadiol	>1	>1
20(*R*)-Protopanaxadiol	>1	>1
Acetohydroxamic acid	0.18	—
Omeprazole	—	0.21

Table 4. Cytotoxicity of 20(*S*)- and 20(*R*)-Ginsenoside R_{g3} and Their Metabolites against Tumor Cell Lines

Compound	IC_{50} (μM)			
	L1210	P388	A549	Me180
Ginsenoside R_{b1}	>100	>100	>100	>100
20(*S*)-Ginsenoside R_{g3}	47	58	>100	>100
20(*R*)-Ginsenoside R_{g3}	>100	>100	>100	>100
20(*S*)-Ginsenoside R_{h2}	22	33	31	28
20(*R*)-Ginsenoside R_{h2}	>100	>100	>100	>100
20(*S*)-Protopanaxadiol	18	33	28	28
20(*R*)-Protopanaxadiol	>100	>100	>100	>100

Based on these findings, we suggest the following metabolic pathway of ginsenosides R_{b1} and R_{b2} (Chart 1). If ginsenosides R_{b1} and R_{b2} are orally administered, they could be transformed to ginsenoside R_{g3} in the stomach. This ginsenoside R_{g3}, which is in Red Ginseng as well as metabolized from ginsenosides R_{b1} and R_{b2} in the stomach, should be metabolized to ginsenoside R_{h2} or 20(*S*)-protopanaxadiol in the human intestine. However, if orally administered ginsenosides R_{b1} and R_{b2} are not transformed to ginsenoside R_{g3} in the stomach, they should be metabolized to IH 901 or 20(*S*)-protopanaxadiol in the human intestine.

To understand what the active compounds of ginsenosides and ginseng extract are when they are orally administered in humans, we measured some biological activities, anti-HP, H^+/K^+ ATPase-inhibitory, and cytotoxic activity against tumor cell lines of ginsenoside R_{g3} and its metabolites. We

found that the cytotoxicity of ginsenosides against tumor cell lines was increased when 20(*S*)-ginsenoside R_{g3} was metabolized to 20(*S*)-ginsenoside R_{h2} or 20(*S*)-protopanaxdiol by human intestinal microflora. Anti-HP activity was also increased when ginsenoside R_{g3} was metabolized to protopanaxadiol. Furthermore, ginsenoside R_{h2} inhibited H^+/K^+ ATPase more potently than ginsenoside R_{g3}. These results suggest that the natural glycosides ginsenosides R_{b1} and R_{g3} are prodrugs, which can be transformed to active compounds by intestinal microflora. Finally, we believe that 20(*S*)-ginsenoside R_{h2} and 20(*S*)-protopanaxadiol transformed from ginseng saponins could play an important role in the antitumor activity, and that protopanaxadiol contained in ginseng extract could inhibit HP growth, while ginsenoside R_{g3} cannot be transformed to protopanaxadiol in the stomach.

Acknowledgement This work was supported by The National Agricultural Cooperative Federation Grant (2001).

REFERENCES

1) Kobashi K., Akao T., *Bioscience Microflora*, **16**, 1—7 (1997).
2) Kim D.-H., Yu K.-W., Bae E.-A., Park H.-J., Choi J.-W., *Biol. Pharm. Bull.*, **21**, 360—365 (1998).
3) Nagai M., Sanada S., Tanaka N., Tanaka O., Shibata S., *Chem. Pharm. Bull.*, **20**, 1212—1216 (1972).
4) Karikura M., Miyaze T., Tanizawa H., Taniyama T., Takino Y., *Chem. Pharm. Bull.*, **38**, 2357—2361 (1991).
5) Wu J. Y., Gardner B. H., Murphy C. L; Seals J. R., Kensil C. R., Recchia J., Beltz G. A., Newman G. W., Newman M. J., *J. Immunol.*, **148**, 1519—1525 (1992).
6) Sato K., Mochizuki M., Saiki I., Yoo Y. C., Samukawa K., Azuma I., *Biol. Pharm. Bull.*, **17**, 635—639 (1994).
7) Mochizuki M., Yoo Y. C., Matsuzawa K., Sato K., Saiki I., Tono-oka S., Samukawa K., Azuma I., *Biol. Pharm. Bull.*, **18**, 1197—1202 (1995).
8) Karikura M., Miyase T., Tanizawa H., Taniyama T., Takino Y., *Chem. Pharm. Bull.*, **39**, 400—404 (1991).
9) Kanaoka M., Kato H., Shimada F., Yano S., *Chem. Pharm. Bull.*, **40**, 314—317 (1992).
10) Kanaoka M., Akao T., Kobashi K., *J. Trad. Med.*, **11**, 241—245 (1994).
11) Akao T., Kanaoka M., Kobashi K., *Biol. Pharm. Bull.*, **21**, 245—249 (1998).
12) Akao T., Kida H., Kanaoka M., Hattori M., Kobashi K., *J. Pharm. Pharmacol.*, **50**, 1155—1160 (1998).
13) Bae E.-A., Park S.-Y., Kim D.-H., *Biol. Pharm. Bull.*, **23**, 1481—1485 (2000).
14) Wakabayashi C., Hasegawa H., Murata J., Saiki I., *Oncol. Res.*, **9**, 411—417 (1998).
15) Lee S. J., Sung J. H., Lee S. J., Moon C. K., Lee B. H., *Cancer Lett.*, **144**, 39—43 (1999).
16) Han B. H., Park M. H., Han Y. N., Woo W. S., Sankawa U., Yahara S., Tanaka O., *Planta Medica*, **44**, 146—149 (1982).
17) Kaku T., Kawashima Y., *Arzneim. Forsch. Drug Res.*, **30**, 936—940 (1980)
18) Baek N. I., Kim D. S., Lee Y. H., Park J. D., Lee C. B., Kim S. I., *Arch. Pharm. Res.*, **18**, 164—168 (1995).
19) Bae E.-A., Han M. J., Kim D.-H., *Planta Medica*, **65**, 442—443 (1999).
20) Odake S., Morikawa T., Tsuchiya M., Imamura L., Kobashi K., *Biol. Pharm. Bull.*, **17**, 1329—1332 (1994).
21) Saccomani G., Mukidjam E., *Biochim. Biophys. Acta*, **912**, 63—73 (1987).
22) Carmichael J., DeGreff W. G., Gazdar A. F., Minna J. D., Mitchell J. B., *Cancer Res.*, **47**, 936—940 (1987).
23) Hasegawa H., Sung J.-W., Benno Y., *Planta Medica*, **63**, 436—440 (1997).